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Identification and Characterization for Agonist-induced Internalization of LH/CG and FSH Receptors by Recombinant eCG Mutants

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The glycoprotein hormone family consists of luteinzing hormone (LH), follicle-stimulating hormone (FSH) and thyroid-stimulating hormone (TSH), which are secreted by the pituitary gland in all mammalian species, and chorionic gonadotropin (CG), which is secreted by the placenta only in primates and equids. Equine chorionic gonadotropin (eCG) is a heavily glycosylated glycoprotein composed of non-covalently linked α- and β-subunit. The β-subunit of equine CG is more than 50% carbohydrate and has a single N-linked, and probably eleven O-linked carbohydrate chains. The endocytosis of eCG mediated by the LH/CG receptor and FSH receptor was not studied in detail in a cultured cell lines that express this receptor endogenously.

Three mutants (WT, $\beta\alpha$56, β-Dα, β-Dα56) were constructed by the change in the oligosaccharide chain of tethered myc eCG$\beta\alpha$. And then each mutant was inserted pcDNA3 vectors. Each gene was added *myc-tag* (Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu) between first amino acid and second amino acid of mature protein in eCG β-subunit. And, plasmids were transfected in 60 mm well culture dishes with 8.0 μg using liposome transfection method. To analysis biological activity *in vivo*, two groups of ICR-mice were injected subcutaneously with 10 IU per mice of myc eCG$\beta\alpha$, eCG $\beta\alpha$56 and eCGβ

−Dα56 and then were injected hCG 10 IU. VSVG expressing vectors for rat LH/CG receptor and FSH receptor were tranfected into CHO− K1 cell lines and selected the clonal lines by cAMP analysis. FSH and LH receptors were pre−incubated with 2 μg/mL CypHer 5−labeled antibody and 1 μm Hoechest nuclear stain for 60 min at room temperature. And then, internalization of the receptors was analyzed by the image Xpress^{micro} system (Molecular Device) and In Cell Analyzer (Amersham Biosciences).

The result of biological activities *in vivo* were obtained 13±4.5 (WT; $\beta\alpha$), 14±3.5 ($\beta\alpha$56), 15±2.0 (β−Dα56) and 20±12.5 (control; native) numbers in the ovulated oocytes by the rec−eCG 10 IU injec− tion. Agonist activation of GPCRs was stimulated by FSH and LH/CG receptor cell for 60 min with rec−eCG WT and rec−eCG mutants (0~20 ng/well). And internalization by the time−course was analyzed (0, 5, 15, 30, 50 and 70 min). Nuclei were stained with a blue flourescent dye (Hoechest). Red flourescent granules adjacent to the nuclei were internalized CypHer5−antibody receptor complex. The internalization of these receptors was increased by dose− dependent of rec−eCG. Now we are investigating Western blot analysis and enzyme release of N−linked oligosaccharides *in vitro* of rec−eCG.

Key words) *Glycosylation, N− and O−linked, Internalization and G−Pro− tein Coupled Receptor*